

Charles Quinn (CQ 8940)
Michelle Wu (MW 0768)
GRAHAM CURTIN, P.A.
4 Headquarters Plaza
P.O. Box 1991
Morristown, New Jersey 07962-1991
(973) 292-1700
Attorneys for Plaintiff

**UNITED STATES DISTRICT COURT
DISTRICT OF NEW JERSEY**

_____	X	
	:	
EZ BIO, INC,	:	Civil Action No.
	:	
Plaintiff,	:	
	:	
v.	:	
	:	COMPLAINT
	:	
GENSCRIPT CORPORATION,	:	
	:	
Defendant.	:	
	:	
_____	X	

Plaintiff EZ Bio, Inc., by its undersigned attorneys, as and for its complaint against defendant GenScript Corporation, says:

PARTIES

1. Plaintiff EZ Bio, Inc. ("EZ Bio") is a corporation organized under the laws of the State of New Jersey, and maintains its principal place of business at 1505 Regents Court, Hillsborough, New Jersey 08844.

2. Defendant GenScript Corporation ("GenScript") is a corporation organized under the laws of the State of Delaware, and maintains its principal place of business at 120 Centennial Avenue, Piscataway, New Jersey 08854.

JURISDICTION

3. This is an action for: (i) unfair competition under 15 U.S.C. §1125, (ii) conversion; (iii) breach of contract; and (iv) trademark infringement.

4. This Court has jurisdiction over this action pursuant to 28 U.S.C. §§1331 and 1338(a) and (b), and pursuant to this Court's supplemental jurisdiction under 28 U.S.C. §1367.

BACKGROUND

5. EZ Bio is a biotechnology company that developed an innovative technology known as Universal Rapid Immuno-detection Method ("Technology"). Immuno-detection is a widely used method in the medical and pharmaceutical industries for identifying or quantifying proteins. Traditional immuno-detection methods require multiple steps and a minimum of five hours. EZ Bio's invention has reduced the multiple steps of traditional methods into a single, one-reaction step that reduces the time for immune-detection to less than one hour.

6. EZ Bio owns, and has always owned, all right, title, and interest in and to the Technology.

7. EZ Bio developed a series of products to exploit its Technology, including the One-Step Western Blot Kit (a/k/a Quick Western Blot Kit), the One-Step IHC Kit and the One-Step ELISA Kit (collectively, "the Products"). EZ Bio sells the Products to research institutions, hospitals, and biotech and pharmaceutical companies in the United States and abroad.

8. In connection with the sales of its Products, EZ Bio registered the following trademark with the State of New Jersey. A true copy of the registration is attached hereto as Exhibit A:

ONE STEP
Registration No. 22400
Registered: January 10, 2006
Expires: January 10, 2011
First Use in New Jersey: July 28, 2005
Class: 001 Chemical Products Used in Industries

9. On April 9, 2004, EZ Bio and GenScript executed a Confidentiality Agreement ("Confidentiality Agreement") as a precondition for EZ Bio to disclose to GenScript its confidential and proprietary information relating to the Technology in connection with a potential business relationship between the parties. A true copy of the Confidentiality Agreement is attached hereto as Exhibit B.

10. The Confidentiality Agreement, *inter alia*, obliged GenScript not to use EZ Bio's confidential or proprietary information relating to the Technology "for any purpose whatsoever . . . other than for the purpose of considering the possibility of joint business relationship."

11. With the protection of the Confidentiality Agreement, EZ Bio began to provide certain of its Products and related confidential or proprietary information to GenScript for evaluation.

12. On March 30, 2005, EZ Bio and GenScript executed a Non-Exclusive Distributorship Agreement for GenScript to serve as a non-exclusive distributor of EZ Bio's Quick Western Blot Kits (or One-Step Western Blot Kits). A true copy of the Non-Exclusive Distributorship Agreement ("Distributorship Agreement") is attached hereto as Exhibit C.

13. Article 11 of the Distributorship Agreement prohibits GenScript from using, or permitting others to use, "EZ Bio" or any other trademark or trade name of EZ Bio without the written permission of EZ Bio. Further, Article 11 of the Distributorship Agreements bars GenScript from contesting EZ Bio's exclusive right to any trademark or trade name used or sought by EZ Bio.

14. In addition, Article 12 of the Non-Exclusive Distributorship Agreement prohibits GenScript from modifying any of EZ Bio's Products or any of the trademarks for EZ Bio's Products without the written permission of EZ Bio.

15. Following the execution of the Distributorship Agreement, EZ Bio first delivered Quick (One-Step) Western Blot Kits to GenScript on July 28, 2005. Further deliveries were made at various times in 2005 and 2006 pursuant to GenScript's purchase orders.

16. In March 2006, EZ Bio noticed that GenScript had modified the Quick (One-Step) Western Blot Kits without EZ Bio's consent and posted the modified product on its website in violation of Article 12 of the Distributorship Agreement.

17. On April 5, 2006, Mr. Charles Zou, President of EZ Bio, notified Mr. Frank Zhang, CEO of GenScript, of the violation of the Distributorship Agreement and demanded that GenScript cure this breach and provide a written explanation as to specific changes GenScript had made to EZ Bio's Quick (One-Step) Western Blot Kits.

18. GenScript did not explain how it had modified the Quick (One-Step) Western Blot Kits, and did not cure the aforesaid breaches of its contractual obligations under the Distributorship Agreement. Instead, GenScript stopped placing purchase orders with EZ Bio and began to produce, market and sell the modified version of the One-Step Western Blot Kits and

other related products under, inter alia, the marks One-Step Western Tag Kits and One-Step Elisa GST Detection Kit.

19. GenScript thereafter filed trademark applications for "One Step" on April 6, 2006 (abandoned on August 21, 2007), and "One-Step Western" (Serial No. 77139261), on March 23, 2007. True copies of these applications are attached as Exhibit D.

20. GenScript used and applied for the trademark "One Step" with full knowledge that EZ Bio had previously registered and used such trademark.

21. In addition, GenScript intentionally misrepresented to the United States Patent and Trademark Office ("USPTO") in connection with its "One-Step Western" trademark application that it had first used this mark on June 1, 2005, and had first used it in commerce on October 1, 2005. In fact, GenScript did not use the trademark "One-Step Western" prior to July 28, 2005, when EZ Bio first sold the Products to GenScript.

22. Further, to deceive customers and potential investors regarding the origin of the Technology, GenScript filed a patent application (U.S. Patent Publication No. 20080261199) covering the Technology. GenScript's patent application largely copies and misappropriates a patent application that EZ Bio had filed covering the Technology (U.S. Patent Publication No. 20050214882) and other documents EZ Bio had provided to GenScript.

23. GenScript has made false and misleading representations in its promotional materials to the consumers as to the origin of the Technology. For example, it claims extensively on its website and in its Technical Manuals that the One-Step Western Kit is one of its newly introduced innovative products using a breakthrough immuno-detection technology. Representative copies of GenScript's promotional materials making such false and misleading representations are attached as Exhibit E.

24. GenScript has been selling the converted product based on its misappropriation of the Technology to consumers both in the United States and around the world.

25. On information and belief, GenScript is planning to use the misappropriated Technology to manufacture the modified version of the One-Step Western Kits and related products in Nanjing, China.

26. GenScript is wrongfully using the misappropriated Technology and plaintiff's trademarks causing customer confusion in the marketplace and irreparable harm and damages to EZ Bio.

COUNT I
BREACH OF THE DISTRIBUTORSHIP AGREEMENT

27. Plaintiff repeats the allegations of the preceding paragraphs as if fully set forth at length here.

28. Pursuant to the Distributorship Agreement, defendant GenScript promised not to, without written permission from EZ Bio, contest the right of EZ Bio to the exclusive use of any trademark or trade name used or claimed by EZ Bio, or modify any of EZ Bio's Products.

29. Defendant GenScript's applications for and use of the trademarks "One-Step" and "One-Step Western" as well as GenScript's wrongful modification of EZ Bio's Quick (One-Step) Western Blot Kits, are wholly without the consent of EZ Bio and constitute breach of the Distributorship Agreement. These breaches have caused EZ Bio to sustain damages and irreparable harm.

COUNT II
CONVERSION

30. Plaintiff repeats the allegations of the preceding paragraphs as if fully set forth at length here.

31. Defendant GenScript has misappropriated EZ Bio's Technology by filing a patent application covering the Technology based on EZ Bio's confidential information and its Patent Application and has wrongfully converted EZ Bio's proprietary Quick (One-Step) Western Blot Kits by modifying the product for its own benefits.

32. Plaintiff EZ Bio has sustained, and continues to sustain damages as a consequence of defendant's misappropriation, conversion and exploitation of EZ Bio's product, Quick (One-Step) Western Blot Kits.

COUNT III
TRADEMARK INFRINGEMENT

33. Plaintiff repeats the allegations of the preceding paragraphs as if fully set forth at length here.

34. Defendant GenScript deliberately, intentionally and fraudulently applied for and used, without EZ Bio's consent, EZ Bio's trademark "One Step" in connection with the sale and advertisement of the modified One-Step Western Blot Kits within the State of New Jersey and elsewhere, with the intention to deceive the public as to the source, sponsorship and origin of the product, which constitutes trademark infringement and unfair competition in violation of N.J.S.A. 56:3-13.16.

35. Defendant's actions as alleged herein have caused, is causing and will continue to cause damages and irreparable injury to EZ Bio.

COUNT IV
UNFAIR COMPETITION UNDER THE LANHAM ACT

36. Plaintiff repeats the allegations of the preceding paragraphs as if fully set forth at length here.

37. Defendant GenScript's infringement of EZ Bio's "One-Step" trademark, and false representations concerning the origin of the converted products violate all norms of acceptable commercial conduct and constitute unfair and unlawful competition in violation of §43(a) of the Lanham Act, 15 U.S.C. §1125. GenScript's conduct has caused, is causing and will continue to cause substantial injury to EZ Bio, including loss of customers, dilution of goodwill, confusion of potential customers, and injury to its reputation.

38. It would be inequitable for defendant GenScript to retain the benefits it unfairly and wrongfully obtained through its infringement of EZ Bio's "One-Step" trademark, and false advertising at the expense of EZ Bio.

WHEREFORE, plaintiff EZ Bio respectfully requests that judgment be entered in its favor and prays:

a. That defendant be preliminarily and permanently enjoined from directly and indirectly manufacturing and selling One Step Western Blot Kits and other related products based on the Technology anywhere in the world and unfairly competing with EZ Bio;

b. That defendant be adjudged to have converted plaintiff's products at plaintiff's expense; to have committed unfair business practices and acts; to have breached its contractual obligations under the Distributorship Agreement; and to have infringed plaintiff's trademark under New Jersey trademark statute;

c. For an accounting of any and all of defendant's profits resulting from its unfair business practices and acts, and that an award of profits be increased as the Court finds to be just under the circumstances;

d. For an award of damages sustained by EZ Bio measured by EZ Bio's actual loss;

e. For punitive damages for defendant's willful, wanton, and intentional acts of unfair competition, conversion and breach of the Distributorship Agreement;

f. For an award of prejudgment and post-judgment interest;

g. For an award of reasonable attorneys' fees and other expenses incurred by EZ Bio in the pursuit of this action; and

h. For costs of suit and such further relief as the Court may deem just and appropriate.

GRAHAM CURTIN, P.A.
Attorneys for Plaintiff

By: _____


CHARLES QUINN (CQ 8940)

February 13, 2009
Morristown, New Jersey

EXHIBIT A

I, THE TREASURER THE STATE OF NEW JERSEY, DO HEREBY
CERTIFY THAT

EZ BIO INC
1505 REGENTS CT
HILLSBOROUGH NJ 08844

DID ON THE 10TH DAY OF JANUARY A.D. 2006 FILE IN THIS
DEPARTMENT

TRADEMARK
MARK REG NUM : 22400

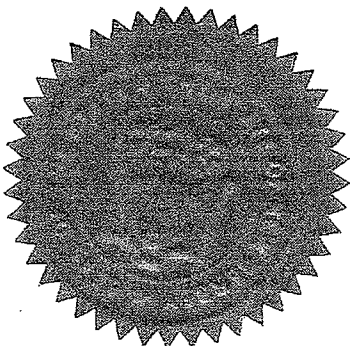
ONE STEP
COMBINES ABOVE WORDS INTO SINGLE TERM TO EXPRESS IMAGE
OF SIMPLE/EASY PROCEEDURE

CLASSIFICATION GROUP : GOODS
CLASS : 001 CHEMICAL PRODUCTS USED IN INDS

REGISTRATION DATE: 01/10/2006
EXPIRATION DATE: 01/10/2011
DATE OF FIRST USE IN NEW JERSEY: 07/28/2005
DATE IN USE ELSEWHERE:

AS BY THE STATUTES OF THIS STATE REQUIRED.

IN TESTIMONY WHEREOF, I HAVE
HEREUNTO SET MY HAND AND AFFIXED
MY OFFICIAL SEAL AT TRENTON, THIS
10TH DAY OF JANUARY
A.D. 2006.



A handwritten signature in cursive script, which appears to read "John E. McCormac".

John E McCormac, CPA
State Treasurer

EXHIBIT B

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PAGE 01

Confidential Agreement.

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CONFIDENTIAL AGREEMENT

THIS AGREEMENT, made as of the 26th day of March, 2004, by and between EZ Bio corporation having offices at 1505 Regents CT, Hillsborough, NJ 08844 (hereinafter referred to as "OWNER"), and GenScript corporation having offices at 120 Centennial Ave, Piscataway, NJ 08854 (hereinafter referred to as "RECIPIENT").

WHEREAS, OWNER possesses certain expertise and information relating to Rapid Immuno-detection techniques and products which are considered trade secret, proprietary and of a confidential nature; and

WHEREAS, OWNER desires to disclose to the RECIPIENT information or samples relating to the rapid immuno-detection techniques and products ("NEW TECHNIQUES") for the purpose to discuss various aspects of a possible joint business relationship.

WHEREAS, the parties agree that the review of the NEW TECHNIQUES will involve the disclosure by OWNER to RECIPIENT of certain confidential and proprietary information and know-how, manufacturing and processing techniques including trade secrets relating to the NEW TECHNIQUES considered confidential by OWNER, which OWNER is not willing to disclose to RECIPIENT except on a confidential basis.

NOW THEREFORE, the parties mutually agree as follows:

1. Confidential and proprietary information and know-how, for the purposes of this Agreement, shall include, without limitation, all scientific, engineering, statistical, commercial, technical, or process data; all information regarding the principle, developing concept, technical strategies and processes; all information regarding existing or potential products, inventions, samples, manufacturing capabilities, all relating to the NEW TECHNIQUES, disclosed or otherwise communicated or transferred by OWNER to RECIPIENT whether directly or indirectly, orally or in writing, in drawings, by way of samples, by observation or otherwise during laboratory or plant visits or in any other way (all such know-how and information being collectively referred to herein as "Information").

- (a) All Information hereafter disclosed by OWNER to RECIPIENT shall be treated by the RECIPIENT as confidential and secret and shall not be revealed or disclosed by the RECIPIENT to any third party whatsoever, and shall not be used for any purpose whatsoever by RECIPIENT other than for the purpose of considering the possibility of joint business relationship.
- (b) The Information may be disclosed to such limited number of officers and employees of the RECIPIENT as may be necessary to implement the purposes described herein, but

Confidential Agreement.

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all such officers and employees shall be directed and required to maintain the Information in confidence.

2. The RECIPIENT shall take all means which are consistent with its current practices for safeguarding confidential information to prevent the Information from being disclosed to any third party or person not entitled thereto.

3. The aforesaid obligation of confidentiality assumed by RECIPIENT shall apply for five (5) years from the date first set forth above.

4. Information disclosed under this Agreement shall not be deemed to be within the foregoing exceptions merely because such information is embraced by more general information in the public domain or in RECIPIENT's possession. In addition, any combination of features shall not be deemed to be within the foregoing exceptions merely because individual features are in the public domain or in RECIPIENT's possession, but only if the combination itself and its principle of operation are in the public domain or in RECIPIENT's possession.

5. All information disclosed by OWNER to RECIPIENT shall be and shall remain the property of the OWNER. To the extent that the Information is in the form of reports or any tangibly recorded data, products or other property, the same shall be returned to the OWNER promptly at its request together with all copies thereof. One copy, however, may be retained in RECIPIENT'S legal files. The RECIPIENT assumes all the liability for damage to, or loss of, any such property while in its possession or subject to its control.

6. OWNER and RECIPIENT acknowledge that the disclosure of information pursuant to this Agreement disclosure does not constitute a sale or an offer for sale of the Information or of any subject matter described therein. Nothing herein contained shall obligate either OWNER or RECIPIENT to enter into any agreement with the other party or into any other commercial agreement, venture or otherwise. Any such agreements may be entered into upon such terms and conditions as the parties may hereafter agree.

7. Nothing herein shall be construed as granting to RECIPIENT any interest or license under any trade secret, patent, patent application, know-how or any copyright heretofore or hereafter granted or filed in which OWNER now has or subsequently obtains any right, title or interest.

8. The parties hereby acknowledge that a breach by either OWNER or RECIPIENT of this Agreement may not be adequately compensated by monetary damages. Accordingly, in the event of such a breach either party recognizes that the other party shall have the immediate right to secure an order from any competent court enjoining such breach. This undertaking shall be enforceable without prejudice to any other rights the parties have in law or under this Agreement.

9. This Agreement constitutes the entire Agreement between the parties and supersedes all written or oral prior agreements or understandings with respect to the subject matter hereof. No variation or modification of the terms and provisions of this Agreement shall be valid unless they are provided in writing and signed by authorized representatives of the parties hereto.

10. This Agreement shall be governed by and interpreted under the laws of the State of New Jersey, and shall be binding upon the parties and their respective successors.

IN WITNESS WHEREOF, the parties have executed this Agreement as of _____, 2004.

Agreed and accepted for EZ Bio corporation

By: _____

Name: Charles Zen

Title: president

4/9/04

Agreed and accepted for GenScript Corporation
[RECIPIENT'S FULL CORPORATE NAME]

By: _____

Name: Frank Zhang

Title: CEO

FRANK ZHANG

EXHIBIT C

NON-EXCLUSIVE DISTRIBUTORSHIP AGREEMENT

This Non-Exclusive Distributorship Agreement ("Agreement"), made and effective this April, 01 2005, by and between EZ Bio Inc. ("Manufacturer") and GenScript Corporation ("Distributor").

Manufacturer desires to appoint Distributor, and Distributor desires to accept appointment, as a distributor of Manufacturer's products as set forth herein.

NOW, THEREFORE, in consideration of the mutual agreements promises set forth herein, the parties agree as follows:

1. Rights Granted.

Manufacturer hereby grants to Distributor a non-exclusive right, on the terms and conditions contained below, to purchase, inventory, promote, repackage and resell "Manufacturer's Products" (as defined below) including using the product's name and information for reselling purpose only. Nothing herein shall prevent or prohibit Manufacturer from selling any of Manufacturer's Products to other customers, but for the same products selling to Distributor, Manufacture agree that directly selling to the following customers: Any distributor, dealer that orders with price equal to or great than \$1500 per one-liter set (good for 20 kits of 50ml-kit); any end user (person, firm or organization) that orders with price equal to or great than \$150 per kit. Distributor respects Manufacturer's right on developing the product, but based on marketing needs, Distributor may claim that Distributor has developed the products.

2. Products.

As used in this Agreement, the term "Manufacturer's Products" shall mean the products, related service parts and accessories manufactured and/or sold by Manufacturer as follows: Quick Western Blot Kit.

3. Terms of Sale.

All sales of Manufacturer's Products to Distributor shall be made pursuant to this Agreement at such prices and on such terms as Manufacturer shall establish from time to time on at least thirty (30) days notice. All prices are FOB Manufacturer's plant, \$1500 per one-liter set (good for 20 kits of 50ml-kit). Manufacturer agrees to properly pack all items for shipment. Risk of loss due to damage or destruction of Manufacturer's Products shall be borne by Distributor after delivery to the carrier for shipment. The shipper will be selected by Manufacturer unless Distributor requests a reasonable alternative. All orders are subject to acceptance by Manufacturer. Except as otherwise expressly agreed by Manufacturer in advance, this Agreement shall control all aspects of the dealings between Manufacturer and Distributor with respect to the Manufacturer's Products and any additional or different terms in any Distributor order are hereby rejected.

4. Payment.

Distributor shall pay 30% of each order in front when order is placed and the rest amount will due when placing the second order. If no further new order is placed, the balance will be paid within 60 days after stoping the order. Manufacturer may or may not impose a late payment charge of one percent (1%) per month on overdue amounts.

5. Marketing Policies.

Distributor will at all times maintain adequate inventories of Manufacturer's Products and will promote vigorously and effectively the sale of Manufacturer's Products through all channels of distribution prevailing in Distributor's "primary marketing area" (as defined below), in conformity with Manufacturer's established marketing policies and programs. Distributor will use its best efforts to sell Manufacturer's Products to aggressive, reputable, and financially responsible dealers providing satisfactory consumer service throughout Distributor's primary marketing area. Distributor is authorized to enter into written agreements with its dealers relating to the purchase, resale and service of Manufacturer's Products on forms approved by Manufacturer for this purpose. Distributor acknowledges its intent to concentrate its marketing of the Manufacturer's Products in its "primary marketing area," including USA.

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6. Advertising Policies.

Manufacturer will cooperate with Distributor and its dealers in providing for continuous and effective advertising and promotion of Manufacturer's Products throughout Distributor's principal marketing area, and Distributor agrees at Distributor's expense to participate in, actively promote and faithfully comply with the terms and conditions of such cooperative advertising and merchandising programs as Manufacturer may establish and offer to Distributor from time to time.

7. Product Warranty Policies.

In the event that any of Manufacturer's Products are proved to Manufacturer's satisfaction to have been defective at time of sale to Distributor, Manufacturer will make an appropriate adjustment in the original sales price of such product or, at Manufacturer's election, replace the defective product. Manufacturer makes no warranty to Distributor with respect to the products repackaged by Distributor. MANUFACTURER MAKES NO WARRANTY TO DISTRIBUTOR WITH RESPECT TO THE PRODUCTS, EITHER EXPRESS OR IMPLIED, INCLUDING, WITHOUT LIMITATION, THE IMPLIED WARRANTIES OF MERCHANTABILITY OR FITNESS FOR A PARTICULAR PURPOSE.

8. Indemnification.

A. Manufacturer agrees to protect Distributor and hold Distributor harmless from any loss or claim arising out of inherent defects in any of Manufacturer's Products existing at the time such product is sold by Manufacturer to Distributor, provided that Distributor gives Manufacturer immediate notice of any such loss or claim and cooperates fully with Manufacturer in the handling thereof.

B. Distributor agrees to protect Manufacturer and hold Manufacturer harmless from any loss or claim arising out of the negligence of Distributor, Distributor's agents, employees or representatives in the installation, use, sale or servicing of Manufacturer's Products or arising out of any representation or warranty made by Distributor, its agents, employees or representatives with respect to Manufacturer's Products that exceeds Manufacturer's limited warranty. Further, in the event that any of Distributor's dealers shall, with respect to any of Manufacturer's Products purchased from Distributor, fail to discharge the dealer's obligations to the original consumer pursuant to the terms and conditions of Manufacturer's product warranty and consumer service policies, Distributor agrees to discharge promptly such unfulfilled obligations.

9. Order Processing and Returns.

A. Manufacturer will employ its best efforts to fill Distributor's orders promptly on acceptance, but reserves the right to allot available inventories among distributors at its discretion.

B. Except for Manufacturer's products that are defective at the time of sales to Distributor, Manufacturer shall not be obligated to accept any of Manufacturer's Products that are returned. In the event such returns are accepted, Manufacturer may impose a reasonable restocking charge.

10. Financial Policies.

Distributor acknowledges the importance to Manufacturer of Distributor's sound financial operation and Distributor expressly agrees that it will:

A. Maintain and employ in connection with Distributor's business and operations under this Agreement such working capital and net worth as may be required to enable Distributor properly and fully to carry out and perform all of Distributor's duties, obligations and responsibilities under this Agreement;

B. Pay promptly all amounts due Manufacturer in accordance with terms of sale extended by Manufacturer from time to time;

C. Furnish Manufacturer with financial statements in such form as Manufacturer may reasonably require from time to time for credit purposes; and

D. Furnish, at Manufacturer's request, a detailed reconciliation of Manufacturer's statements of account with Distributor's records, listing all differences, and showing net amount Distributor acknowledges to be due Manufacturer.

In addition to any other right or remedy to which Manufacturer may be entitled, shipments may be suspended at Manufacturer's discretion in the event that Distributor fails to promptly and faithfully discharge each and every obligation in this Section.

11. Use of Manufacturer's Name and Distributor's Name.

Distributor will not use, authorize or permit the use of, the name "EZ Bio" or any other trademark or trade name owned by Manufacturer as part of its firm, corporate or business name in any way without written permission from Manufacturer. Distributor shall not contest the right of Manufacturer to exclusive use of any trademark or trade name used or claimed by Manufacturer. Distributor may, subject to Manufacturer's policies regarding reproduction of same, utilize Manufacturer's name, trademarks or logos in advertising on stationery and business cards.

Manufacturer will not use, authorize or permit the use of, the name "GenScript Corporation" or any other trademark or trade name owned by Distributor as part of its firm, corporate or business name in any way without written permission from Distributor.

12. Relationship of the Parties.

The relationship between Manufacturer and Distributor is that of vendor and vendee. Distributor, its agents and employees shall, under no circumstances, be deemed employees, agents or representatives of Manufacturer. Distributor will not modify any of Manufacturer's Products and any of the names of Manufacturer's Products without written permission from Manufacturer. Neither Distributor nor Manufacturer shall have any right to enter into any contract or commitment in the name of, or on behalf of the other, or to bind the other in any respect whatsoever.

13. Term and Termination.

Unless earlier terminated as provided below, the term of this Agreement shall commence April, 01 2005 and shall continue until April, 01 2015. At the end of the term, the Agreement shall continue until terminated by either party on at least thirty (30) days prior notice.

A. Manufacturer may terminate at any time by written notice given to Distributor not less than sixty (60) days prior to the effective date of such notice in the event Manufacturer decides to terminate all outstanding distributor agreements for Manufacturer's Products and to offer a new or amended form of distributor agreement.

B. Manufacturer may terminate this Agreement upon notice to Distributor, upon any of the following events: (1) failure of Distributor to fulfill or perform any one of the duties, obligations or responsibilities of Distributor in this Agreement, which failure is not cured with five (5) days notice from Manufacturer; (2) any assignment or attempted assignment by Distributor of any interest in this agreement or delegation of Distributor's obligations without Manufacturer's written consent; (3) any sale, transfer or relinquishment, voluntary or involuntary, by operation of law or otherwise, of any material interest in the direct or indirect ownership or any change in the management of Distributor; (4) failure of Distributor for any reason to function in the ordinary course of business; (5) conviction in a court of competent jurisdiction of Distributor, or a manager, partner, principal officer or major stockholder of Distributor for any violation of law tending, in Manufacturer's opinion, to affect adversely the operation or business of Distributor or the good name, goodwill, or reputation of Manufacturer, products of Manufacturer, or Distributor; or (6) submission by Distributor to Manufacturer of false or fraudulent reports or statements, including, without limitation, claims for any refund, credit, rebate, incentive, allowance, discount, reimbursement or other payment by Manufacturer.

14. Obligations on Termination.

On termination of this Agreement, Distributor shall cease to be an authorized distributor of Manufacturer and:

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A. All amounts owing by Distributor to Manufacturer shall, notwithstanding prior terms of sale, become immediately due and payable;

B. All unshipped orders shall be cancelled without liability of either party to the other;

C. Distributor will resell and deliver to Manufacturer on demand, free and clear of liens and encumbrances, such of Manufacturer's Products and materials bearing Manufacturer's name as Manufacturer shall elect to repurchase, at a mutually agree price, but not in excess of Manufacturer's current price to distributors for such products and materials, provided that Manufacturer shall not be obligated to pay Distributor for any item originally provided free of charge; and

D. Neither party shall be liable to the other because of such termination for compensation, reimbursement or damages on account of the loss of prospective profits or anticipated sales, or on account of expenditures, investments, lease or commitments in connection with the business or good will of Manufacturer or Distributor or for any other reason whatsoever growing out of such termination.

15. Use of Name Prohibited.

On termination of this Agreement, Distributor will remove and not thereafter use any sign containing any name or information of Manufacturer's products including, but not limited to, Quick Western Blot Detection kit or Quick WB Detection kit, and will immediately destroy all stationery, advertising matter and other printed matter in its possession or under its control containing such name, or any of Manufacturer's products. Distributor will not at any time after such termination use or permit any name or information of Manufacturer's products to be used in any manner in connection with any business conducted by it or in which it may have an interest, or otherwise whatsoever as descriptive of or referring to anything other than merchandise or products of Manufacturer. Regardless of the cause of termination, Distributor will immediately take all appropriate steps to remove and cancel its listings in website, and other directories, and public records, or elsewhere that contain the names or information of Manufacturer's products

16. Acknowledgments.

Each party acknowledges that no representation or statement, and no understanding or agreement, has been made, or exists, and that in entering into this Agreement each party has not relied on anything done or said or on any presumption in fact or in law, (1) with respect to this Agreement, or to the duration, termination or renewal of this Agreement, or with respect to the relationship between the parties, other than as expressly set forth in this Agreement; or (2) that in any way tends to change or modify the terms, or any of them, of this Agreement or to prevent this Agreement becoming effective; or (3) that in any way affects or relates to the subject matter hereof. Distributor also acknowledges that the terms and conditions of this Agreement, and each of them, are reasonable and fair and equitable.

17. Final Agreement.

This Agreement terminates and supersedes all prior understandings or agreements on the subject matter hereof. This Agreement may be modified only by a further writing that is duly executed by both parties.

18. Assignment.

Neither this Agreement nor any interest in this Agreement may be assigned by Distributor without the prior express written approval of Manufacturer, which may be withheld by Manufacturer at Manufacturer's absolute discretion.

19. No Implied Waivers.

Except as expressly provided in this Agreement, waiver by either party, or failure by either party to claim a default, of any provision of this Agreement shall not be a waiver of any default or subsequent default.

20. Notices.

Any notice required by this Agreement or given in connection with it, shall be in writing and shall be given to the appropriate party by personal delivery or by certified mail, postage prepaid, or recognized overnight delivery services;

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If to Manufacturer:

EZ Bio Inc.

1505 Regents CT, Hillsborough, NJ 08844

If to Distributor:

GeneScript Corporation

120 Centennial Ave, Piscataway, NJ 08854

21. Governing Law.

This Agreement shall be construed and enforced in accordance with the laws of the state of New Jersey.

22. Severability.

If any term of this Agreement is held by a court of competent jurisdiction to be invalid or unenforceable, then this Agreement, including all of the remaining terms, will remain in full force and effect as if such invalid or unenforceable term had never been included.

23. Headings.

Headings used in this Agreement are provided for convenience only and shall not be used to construe meaning or intent.

IN WITNESS WHEREOF, the parties have executed this Agreement as of the date first above written.

EZ Bio Inc.

GeneScript Corporation

By: 

Charles C. Zou
President

3/30/05

By: 

Frank Zhang
CEO

3/30/05

EXHIBIT D

Trademarks > Trademark Electronic Search System (TESS)

TESS was last updated on Sat Dec 13 04:03:19 EST 2008

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Standard Characters Claimed

Mark Drawing Code (4) STANDARD CHARACTER MARK

Serial Number 78856080

Filing Date April 6, 2006

Current Filing Basis 1A

Original Filing Basis 1A

Owner (APPLICANT) GenScript Corporation CORPORATION DELAWARE Suite 105 120 Centennial Ave. Piscataway NEW JERSEY 08854

Type of Mark TRADEMARK

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Word Mark ONE-STEP WESTERN

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Standard Characters Claimed

Mark Drawing Code (4) STANDARD CHARACTER MARK

Serial Number 77139261

Filing Date March 23, 2007

Current Filing Basis 1A

Original Filing Basis 1A

Owner (APPLICANT) GenScript Corporation CORPORATION NEW JERSEY 120 Centennial Ave. Piscataway NEW JERSEY 08854

Attorney of Record Thomas D. Foster

Type of Mark TRADEMARK

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Live/Dead Indicator LIVE

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EXHIBIT E



One-Step Western™ Complete Kit

Developed using our proprietary One-Step Western™ technology, One-step Western™ Complete Kit reduces the total analysis time for a Western blot or Dot blot to just one hour. The kit contains all the necessary reagents, buffers, nitrocellulose membrane and HRP substrate for performing western blot, and compatible with both chemiluminescent substrates and chromogenic substrates, such as DAB and TMB.

Key Features:

- **Easy to perform:** Fewer steps mean fewer chances for human error.
- **High sensitivity:** The sensitivity of the kit is comparable to or better than that of the classical 4.5-hour procedure, depending on the quality and amount of antibodies used.
- **Reproducible results:** The kit produces highly reproducible results.
- **Needs far less optimization** than the classical three-step method.
- **No additional secondary antibody is needed.**

Applications:

One-Step Western™ Kit cuts hours off the following applications. One-Step Western™ Complete Kit provides everything for such operation:

- Protein/antigen detection
- Evaluation of protein expression
- Antibody/antigen titration
- Analysis and comparison of primary antibody activity levels

Example:

1. Western blot detection of housekeeping protein α -tubulin.

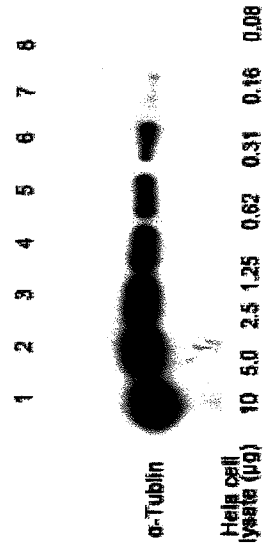


Figure 2. Western blot detection of α -Tubulin using the One-Step Western™ Kit (Cat. No. L00204C) and α -tubulin polyclonal antibody (Abcam, ab4074). 10 μ g, 5.0 μ g, 2.5 μ g, 1.25 μ g, 0.62 μ g, 0.31 μ g, 0.16 μ g, and 0.08 μ g of Hela cell lysates (BD Biosciences, 611449) were loaded into Lane 1, Lane 2, Lane 3, Lane 4, Lane 5, Lane 6, Lane 7, and Lane 8, respectively. The blot was developed with the LumiSensor™ Chemiluminescent HRP Substrate included in the kit.

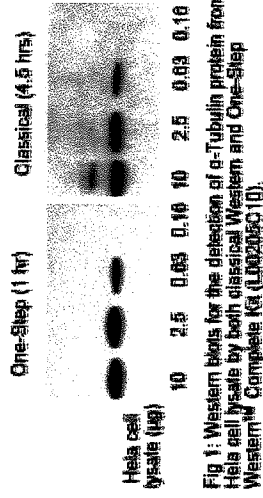


Fig 1: Western blots for the detection of α -Tubulin protein from Hela cell lysate by both classical Western and One-Step Western™ Complete Kit (L00204C10).

Cat. No.	Name	Size	Price	Promotion Price	Quantity
<input type="checkbox"/> L00228	One-Step Western™ Complete Kit (Goat)	1 Kit (10 Assays)	\$275.00	\$195.00	1
<input type="checkbox"/>		5 Kit (50 Assays)	\$962.50	\$818.50	1
<input type="checkbox"/> L00205C	One-Step Western™ Complete Kit (Mouse)	1 Kit (10 Assays)	\$275.00	\$195.00	1
<input type="checkbox"/>		5 Kit (50 Assays)	\$962.50	\$818.50	1
<input type="checkbox"/> L00204C	One-Step Western™ Complete Kit (Rabbit)	1 Kit (10 Assays)	\$275.00	\$195.00	1
<input type="checkbox"/>		5 Kit (50 Assays)	\$962.50	\$818.50	1
<input type="checkbox"/> L00204T	One-Step Western™ Complete Kit Using TMB (Rabbit)	1 Kit (10 Assays)	\$255.00	\$178.50	1
<input type="checkbox"/> L00205T	One-Step Western™ Complete Kit Using TMB (Mouse)	1 Kit (10 Assays)	\$255.00	\$178.50	1
<input type="checkbox"/> L00228T	One-Step Western™ Complete Kit Using TMB (Goat)	1 Kit (10 Assays)	\$255.00	\$178.50	1

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One-Step Western™ Complete Kit (Rabbit)

Kit Description

GenScript's One-Step Western™ Kit yields journal-quality Western or Dot blot results in about one hour. Using GenScript's breakthrough immunodetection technology (patent pending), the kit replaces the classical three-step Western process, which can take nearly five hours. Transfer the proteins from gel to membrane and incubate it in the pretreat solution for five minutes. Then incubate in WB-2 solution with the mixture of primary antibody and WB-1 solution for 40 minutes. Then wash three times for five or ten minutes each. The membrane can then be developed with the HRP substrate. The One-Step Western™ procedure is contrasted with a classical three-step Western at right.

The One-Step Western™ Kit is designed to produce high signal with low background for quick and clear Western analysis of proteins.

Except for basic kits, all other kits contain all the necessary reagents, buffers and materials such as membrane and X-ray films for performing a Western blot, and no additional secondary antibody is needed.

The One-Step Western™ Kit saves precious research time. For added cost-efficiency, we recommend our complete kits, which contain all the reagents, membrane, HRP substrate, and X-ray film necessary for a perfect blot. However, the kit components are also sold separately.

Key Features

- **Easy to perform:** This kit has fewer and simpler steps than other Western kits.
- **Low background:** The kit contains WestClear™ Nitrocellulose Membrane and LumiSensor™ series of Chemiluminescent HRP Substrate Kits, and X-ray film, optimized for low background.
- **Reproducible results:** The kit produces highly reproducible results.
- **No additional secondary antibody** is needed.

Order:

Cat.No.	Name	Size	Price	Quantity
<input type="checkbox"/> L00204C	One-Step Western™ Complete Kit (Rabbit)	1 Kit	\$-275.00 \$ 195.00	1
<input type="checkbox"/> L00204C	One-Step Western™ Complete Kit (Rabbit)	5 Kit	\$-962.50 \$ 818.50	1

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Technical Manual:

- Download PDF file TM0362

Components:

Components	L00228	L00204C	L00204T	L00205C	L00205T	L00228T
Pretreat Solution A	100 ml	100 ml	100 ml	100 ml	100 ml	100 ml
Pretreat Solution B	100 ml	100 ml	100 ml	100 ml	100 ml	100 ml
WB-1 Solution	1 ml	1 ml	1 ml	1 ml	1 ml	1 ml
WB-2 Solution	100 ml	100 ml	100 ml	100 ml	100 ml	100 ml
10X Wash Solution	125 ml	125 ml	125 ml	125 ml	125 ml	125 ml
WestClear™ Nitrocellulose Membrane (0.2 µm, 7.5 x 8 cm)	10 sheets	10 sheets	10 sheets	10 sheets	10 sheets	10 sheets
ChromoSensor™ One Solution TMB Substrate	X	X	60 ml	X	60 ml	60 ml
LumiSensor™ Chemiluminescent HRP Substrate	2 x 15 ml	2 x 15 ml	X	2 x 15 ml	X	X
High-Sensitivity X-Ray Film (5 x 7 inches)	20 sheets	20 sheets	X	20 sheets	X	X
Protocol	1	1	1	1	1	1

PROTOCOL: 20080716033645 (PDF)

MSDS: 20060816160706 (PDF)

Examples:



You are here: [Products](#) » [Immunoassay Products](#) » [Western Technology](#) » [One-Step Western™ Complete Kit](#) » [One-Step Western™ Complete Kit \(Mouse\)](#)

One-Step Western™ Complete Kit (Mouse)

Kit Description

GenScript's One-Step Western™ Kit yields journal-quality Western or Dot blot results in about one hour. Using GenScript's breakthrough immunodetection technology (patent pending), the kit replaces the classical three-step Western process, which can take nearly five hours. Transfer the proteins from gel to membrane and incubate it in the pretreat solution for five minutes. Then incubate in WB-2 solution with the mixture of primary antibody and WB-1 solution for 40 minutes. Then wash three times for five or ten minutes each. The membrane can then be developed with the HRP substrate. The One-Step Western™ procedure is contrasted with a classical three-step Western at right.

The One-Step Western™ Kit is designed to produce high signal with low background for quick and clear Western analysis of proteins.

Except for basic kits, all other kits contain all the necessary reagents, buffers and materials such as membrane and X-ray films for performing a Western blot, and no additional secondary antibody is needed.

The One-Step Western™ Kit saves precious research time. For added cost-efficiency, we recommend our complete kits, which contain all the reagents, membrane, HRP substrate, and X-ray film necessary for a perfect blot. However, the kit components are also sold separately.

Key Features

- **Easy to perform:** This kit has fewer and simpler steps than other Western kits.
- **Low background:** The kit contains WestClear™ Nitrocellulose Membrane and LumiSensor™ series of Chemiluminescent HRP Substrate Kits, and X-ray film, optimized for low background.
- **Reproducible results:** The kit produces highly reproducible results.
- **No additional secondary antibody** is needed.

Order:

Cat.No.	Name	Size	Price	Quantity
<input type="checkbox"/> L00205C	One-Step Western™ Complete Kit (Mouse)	1 Kit	\$275.00 \$ 195.00	1
<input type="checkbox"/> L00205C	One-Step Western™ Complete Kit (Mouse)	5 Kit	\$962.50 \$ 818.50	1

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Technical Manual:

- Download PDF file **TM0352**

Components:

Components	L00228	L00204C	L00204T	L00205C	L00205T	L00228T
Pretreat Solution A	100 ml	100 ml	100 ml	100 ml	100 ml	100 ml
Pretreat Solution B	100 ml	100 ml	100 ml	100 ml	100 ml	100 ml
WB-1 Solution	1 ml	1 ml	1 ml	1 ml	1 ml	1 ml
WB-2 Solution	100 ml	100 ml	100 ml	100 ml	100 ml	100 ml
10X Wash Solution	125 ml	125 ml	125 ml	125 ml	125 ml	125 ml
WestClear™ Nitrocellulose Membrane (0.2 µm, 7.5 x 8 cm)	10 sheets	10 sheets	10 sheets	10 sheets	10 sheets	10 sheets
ChromoSensor™ One Solution TMB Substrate	X	X	60 ml	X	60 ml	60 ml
LumiSensor™ Chemiluminescent HRP Substrate	2 x 15 ml	2 x 15 ml	X	2 x 15 ml	X	X
High-Sensitivity X-Ray Film (5 x 7 inches)	20 sheets	20 sheets	X	20 sheets	X	X
Protocol	1	1	1	1	1	1

PROTOCOL: 20080716033645 (PDF)
 MSDS: 20080818160706 (PDF)

Examples:

One-Step Western™ Kit



Technical Manual No. 0352

Version 08192008

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IV	Related Products	3
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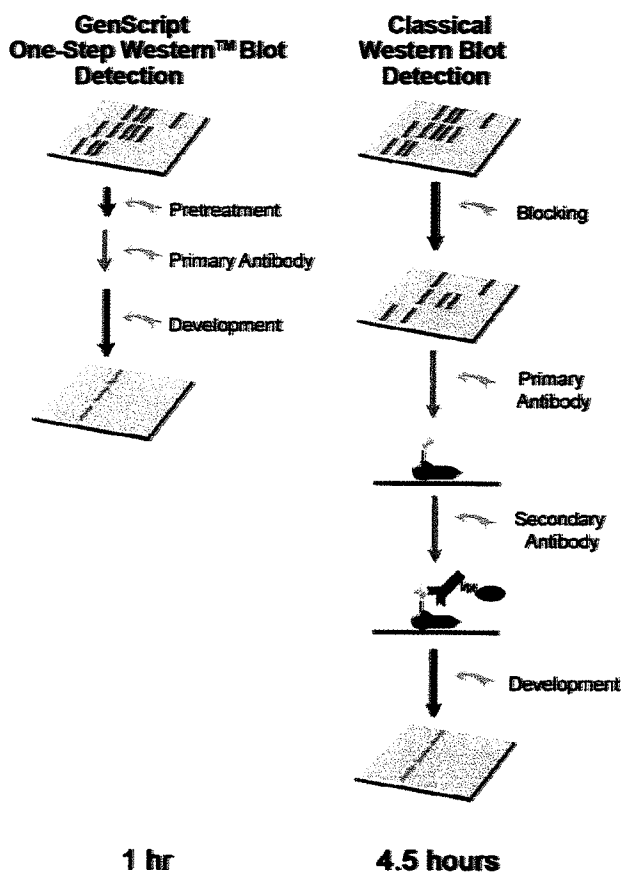
I. DESCRIPTION

GenScript's One-Step Western™ Kit yields journal-quality Western or Dot blot results in about one hour. Using GenScript's breakthrough immunodetection technology (patent pending), the kit replaces the classical three-step Western process, which can take nearly five hours. Transfer the proteins from gel to membrane and incubate it in the pretreat solution for five minutes. Then incubate in WB-2 solution with the mixture of primary antibody and WB-1 solution for 40 minutes. Then wash three times for five or ten minutes each. The membrane can then be developed with the HRP substrate. The One-Step Western™ procedure is contrasted with a classical three-step Western at right.

The One-Step Western™ Kit is designed to produce high signal with low background for quick and clear Western analysis of proteins.

Except for basic kits, all other kits contain all the necessary reagents, buffers and materials such as membrane and X-ray films for performing a Western blot, and no additional secondary antibody is needed.

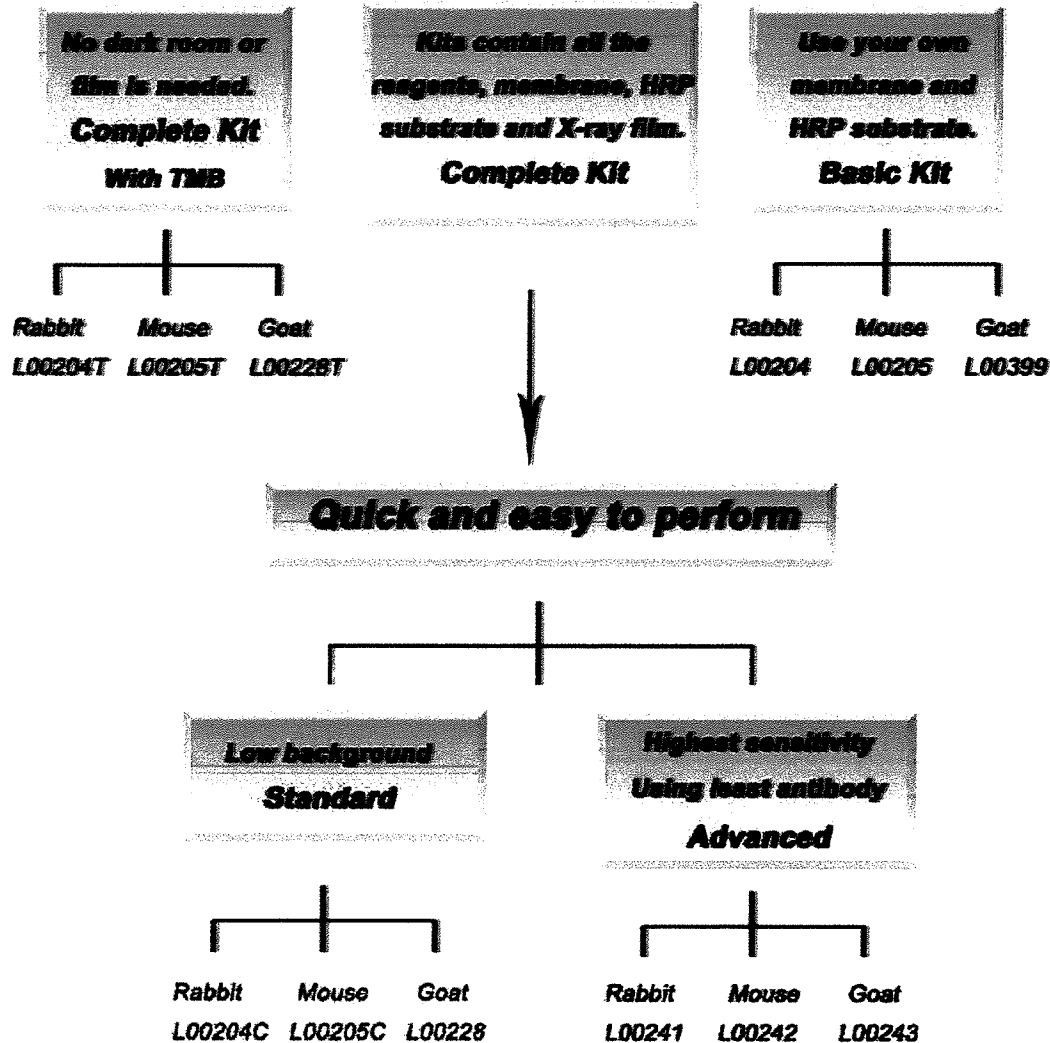
The One-Step Western™ Kit saves precious research time. For added cost-efficiency, we recommend our complete kits, which contain all the reagents, membrane, HRP substrate, and X-ray film necessary for a perfect blot. However, the kit components are also sold separately.



Overview of Western Procedures



II. ONE-STEP WESTERN KIT SELECTION GUIDE

No Secondary Antibody Is Needed for Any of These Kits

Sensitivity	HIGH	HIGHEST
Amount of antibody	2 - 10 µg	0.5 - 2.5 µg
Total time needed	1.25 hrs	1.25 hrs



III. KIT CONTENTS

Each kit contains enough reagents for ten minigel (7.5 x 8 cm) Western blots.

Kit Components	Basic Kits	Complete Kits with TMB	Complete Kits	
			Standard	Advanced
Pretreat Solution A	100 ml	100 ml	100 ml	100 ml
Pretreat Solution B	100 ml	100 ml	100 ml	100 ml
WB-1 Solution	1 ml	1 ml	1 ml	1 ml
WB-2 Solution	100 ml	100 ml	100 ml	100 ml
10X Wash Solution	125 ml	125 ml	125 ml	125 ml
WestClear™ Nitrocellulose Membrane (0.2 µm, 7.5 x 8 cm)	X	10 sheets	10 sheets	10 sheets
ChromoSensor™ One Solution TMB Substrate	X	60 ml	X	X
LumiSensor™ Chemiluminescent HRP Substrate*	X	X	2 x 15 ml	10 + 20 ml
High-Sensitivity X-Ray Film (5 x 7 inches)	X	X	20 sheets	20 sheets
Protocol	1	1	1	1

* Standard Complete Kits and Advanced Complete Kits include LumiSensor™ and LumiSensor™ Super Chemiluminescent HRP Substrate, respectively.

In some rare cases, the primary antibody (including some antibodies against phosphoproteins) may not be compatible with Pretreat Solution A, resulting in very high background. For these cases, GenScript provides an alternate pretreat solution A (Pretreat A-b) to solve this problem. This solution (GenScript, M01052) is available separately. Customers can also use the GenScript Quick Block Optimization Kit to find the best blocking reagent for their specific projects.

Pretreat A-b	M01052	100 ml
Western Quick Block Optimization Kit	L00278	1 kit

IV. RELATED PRODUCTS

• WestClear™ Nitrocellulose Membrane (0.2 µm)	L00224A60
• ChromoSensor™ One Solution TMB Substrate	L00222V60
• LumiSensor™ Chemiluminescent HRP Substrate Kit	L00221V60
• LumiSensor™ Plus Chemiluminescent HRP Substrate Kit	L00225
• LumiSensor™ Super Chemiluminescent HRP Substrate Kit	L00354
• 10X Wash Solution	MB01011
• Pretreat Solutions (A + B)	M01013
• Pretreat A-b	M01052
• GenScript Dot Blot Box	M00108
• Western Quick Block Optimization Kit	L00278
• High-Sensitivity X-Ray Film (5 x 7 inches, 20 sheets)	L00358
• Western Blot Box	M00100

V. KEY FEATURES

- ◆ **Easy to perform:** This kit has fewer and simpler steps than other Western kits.
- ◆ **Low background:** The kit contains WestClear™ Nitrocellulose Membrane and LumiSensor™ series of Chemiluminescent HRP Substrate Kits, and X-ray film, optimized for low background.
- ◆ **Reproducible results:** The kit produces highly reproducible results.



- ◆ No additional secondary antibody is needed.

VI. STORAGE

Store WestClear™ Nitrocellulose Membrane at room temperature. Store the rest of the kit at 4°C. It will remain stable for six months. **Do not freeze the kit or any of its components.**

VII. ONE-STEP WESTERN™ PROTOCOL

This procedure is optimized for a sheet of 7.5 x 8.0 cm membrane, but reagent volumes can be scaled according to the size of the membrane used.

Reagents not provided:

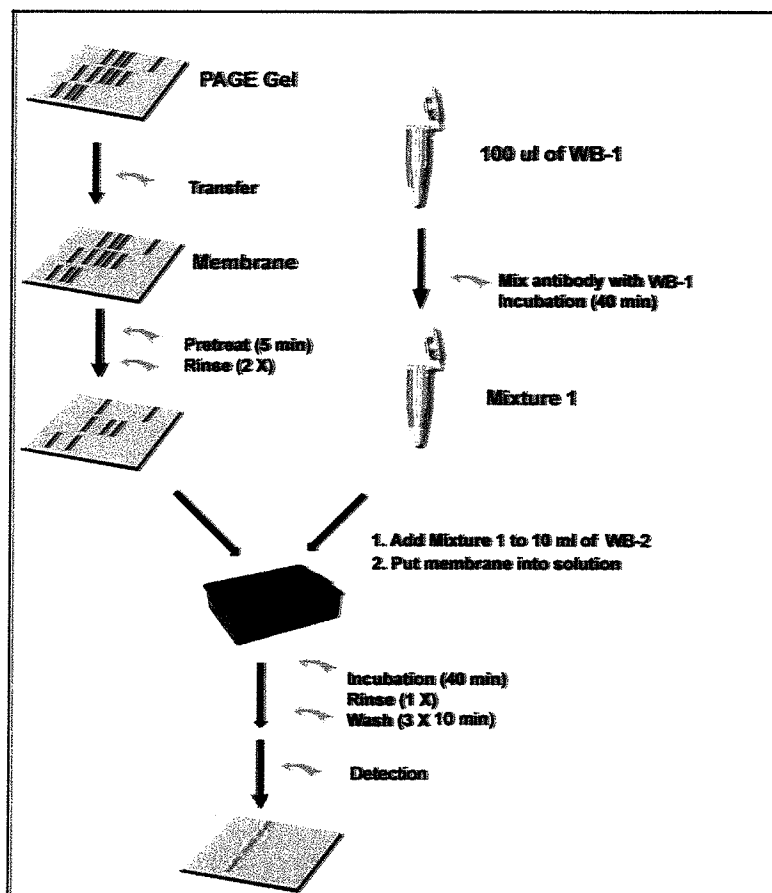
Purified primary antibodies: Affinity-purified antibodies are recommended. Further optimization may be needed if the serum containing the antibody is to be used.

Before use, prepare the following:

1X wash solution: Dilute 12.5 ml of 10X wash solution with 112.5 ml of distilled or filtered water to make 125 ml of 1X wash solution. If any precipitate forms in the 10X wash solution during storage, incubate the bottle in a warm or hot water bath (up to 50°C) with occasional mixing until all the precipitate disappears. Dilute the buffer with ddH₂O to 1X and store it at 4°C. Use 15 ml of 1X wash solution for each rinse and 20 ml of 1X wash solution for each wash.

Western blot procedure:

1. Prepare Mixture 1



Before or during protein transfer, prepare mixture 1 by mixing primary antibody with WB-1 in a microcentrifuge tube. Vortex mixture 1 gently for a few seconds and centrifuge briefly to collect the solution in the bottom of the tube. Incubate mixture 1 at room temperature (RT) for at least 40 minutes.

Mixture 1 Preparation	L00204, L00205, and L00399	L00204T, L00205T, and L00228T	L00204C, L00205C, and L00228	L00241, L00242, and L00243
WB-1 Solution	20 - 100 μ l	50 - 100 μ l	20 - 100 μ l	10 - 50 μ l
Primary Antibody*	2 - 10 μ g	5 - 10 μ g	2 - 10 μ g	0.5 - 2.5 μ g
Ratio of WB-1: Antibody	10 μ l : 1 μ g	10 μ l : 1 μ g	10 μ l : 1 μ g	20 μ l : 1 μ g
For Antibody without Known Titer	Mix 5 μ g of Ab with 50 μ l WB-1	Mix 5 μ g of Ab with 50 μ l WB-1	Mix 5 μ g of Ab with 50 μ l WB-1	Mix 1 μ g of Ab with 20 μ l WB-1

* Refer to manufacturer's recommendations when using appropriate amounts of antibody. With One-Step Advanced Western Kits, use 1/4 to 1/2 of the recommended amount. For antibodies without known titers, start with 1 μ g for Advanced Western Kits and 5 μ g for other One-Step Western™ Kits.

**2. Pre-Treat Membrane**

Just before the protein transfer from gel to membrane is complete, mix 10 ml of pretreat solution A with 10 ml of pretreat solution B in a plastic container (Western wash box (GenScript, M00100)) to make the pretreat solution. Always prepare and use fresh mixture. Place the membrane directly in the pretreat solution mixture and incubate on a shaker for five minutes at RT. After incubation, rinse the membrane twice with 15 ml of 1X wash solution.

3. Final Incubation of Pre-Treated Membrane

- a. Add mixture 1 to 10 ml of WB-2 in a Western blot box and mix well. Incubate the membrane in this solution (WB-2 containing mixture 1) on a shaker at RT for 40 minutes. This mixture (WB-2 containing mixture 1) may be recovered and reused up to three times if stored at 4°C. However, this may cause variations to arise due to changes in antibody concentration and carryover contamination.
- b. Rinse the membrane once with 15 ml of 1X wash solution. Wash the membrane on a shaker three times for ten minutes each with 20 ml of 1X wash solution. When using TMB substrate, wash the membrane three times for just five minutes each with 20 ml of 1X wash solution. **Use a clean container for each wash step to avoid carryover contamination and to reduce background.**

4-1. Signal Development with Chemiluminescent HRP Substrate

- a. When using LumiSensor™ HRP Substrate, mix 1.5 ml of reagent A with 1.5 ml of reagent B by vortexing for a few seconds to make the working solution. When using LumiSensor™ Super Chemiluminescent HRP Substrate, mix 1.0 ml of reagent A with 2.0 ml of reagent B by vortexing for a few seconds to make the working solution. 0.05 ml of the working solution is sufficient to cover 1 cm² of membrane. When protected from light, the working solution (A+B) remains stable for several hours at room temperature.

Summary of Working Solution Preparation: 0.05 ml Is Needed per cm² of Membrane

Working Solution Preparation	L00204C, L00205C, and L00228	L00241, L00242, and L00243
Reagent A	1.5 ml	1.0 ml
Reagent B	1.5 ml	2.0 ml
Total Volume	3.0 ml	3.0 ml

- b. Drain the excess wash solution from the membrane by holding the membrane vertically with forceps and touching the edge against a tissue. Place the membrane on clean, flat surface, and cover the membrane with working solution.
- c. Incubate for **three minutes** at room temperature. Place the membrane on a soft, clean tissue. Use another tissue to remove excess working solution. Wrap the membrane in a clean piece of plastic film.
- d. Expose to a sheet of film for 30 seconds and then develop. Repeat with different exposure times to find the best results.

4-2. Signal Development with TMB Substrate

- a. ChromoSensor™ One Solution TMB Substrate is a ready-to-use working solution, and 0.1 ml is sufficient to cover 1 cm² of membrane. Drain the excess wash solution from the membrane by holding the membrane vertically with forceps and touching the edge against a tissue. Place the membrane on a clean plate and cover it with TMB.
- b. Incubate for 5 to 10 minutes at room temperature until the desired color intensity is reached. Stop the reaction by rinsing the membrane three times for thirty seconds each in 20 ml of deionized water.
- c. Drain off the excess water and transfer the membrane to a piece of paper towel. Air-dry the membrane in a dark place.



VIII. EXAMPLES

Comparison of the two One-Step Western™ Blot Kits of different sensitivities using monoclonal antibodies:

Two similar blots were processed with the same procedures using different One-Step Western™ Kits: Standard (L00205C) and Advanced (L00242). 10 µg and 2.5 µg of THE™ Anti-GST Monoclonal Antibody (Mouse) (GenScript, A00865), respectively, were used with these two kits to detect GST protein.

The results are shown in Figure 1.

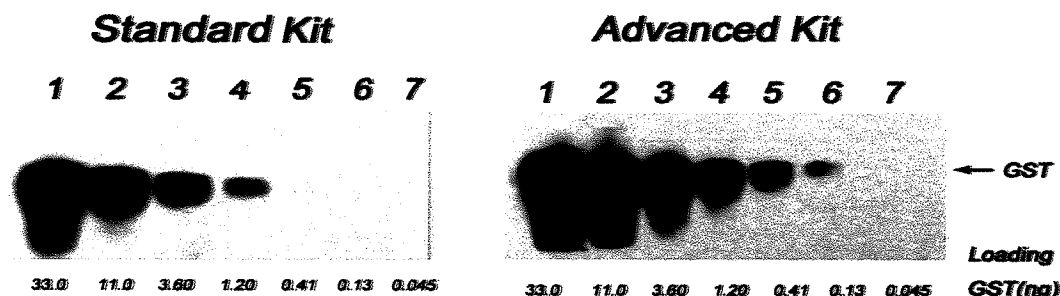


Figure 1. Western blots for the detection of GST protein by One-Step Western™ using different One-Step Western™ Kits: Standard (L00205C) and Advanced (L00242). 33.0, 11.0, 3.60, 1.20, 0.41, 0.13 and 0.045 ng of GST protein were loaded into Lanes 1, 2, 3, 4, 5, 6, and 7 respectively.

Comparison of the two One-Step Western™ Blot Kits of different sensitivities using polyclonal antibodies:

Two similar blots were processed with the same procedures using different One-Step Western™ Kits: Standard (L00204C) and Advanced (L00241). 10 µg and 2.5 µg of Rabbit Anti-GST-tag Polyclonal Antibody (GenScript, A00097), respectively, were used with the two kits to detect GST protein.

The results are shown in Figure 2.

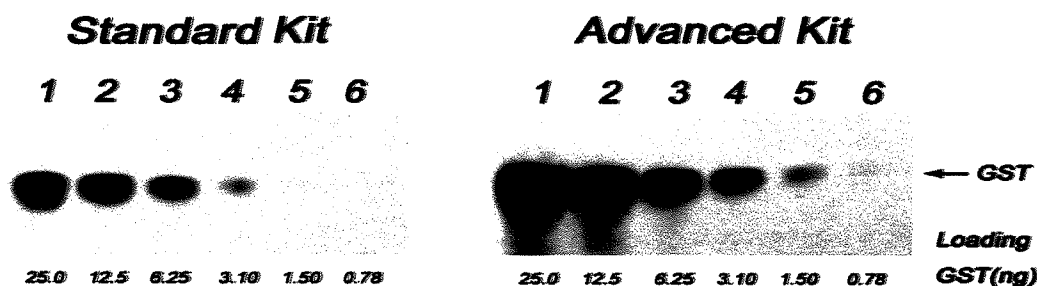


Figure 2. Western blots for the detection of GST protein by One-Step Western™ using different One-Step Western™ Kits: Standard (L00204C) and Advanced (L00241). 25.0, 12.5, 6.25, 3.10, 1.50 and 0.78 ng of GST protein were loaded into Lanes 1, 2, 3, 4, 5, and 6 respectively.

**IX. TROUBLESHOOTING**

Problem	Probable Cause	Solution
The signal is weak or invisible.	Too little protein is loaded.	Load more protein(s) onto the SDS-PAGE gel.
	There is poor transfer efficiency.	Optimize the transfer time and/or the electrical current. Make sure that there are no air bubbles between the membrane and the gel.
	The primary antibody has a low affinity for the antigen.	Increase the incubation time of the membrane in WB-2 containing mixture 1. Increasing antibody concentration can also improve signal.
	The primary antibody has a low affinity for the antigen.	Reducing wash time can increase the signal for low-affinity antibody. Instead of wash for 3 x 10 min, wash for 3 x 5 min to increase signal.
There is high background.	Too much primary antibody is used.	Reduce the amount of primary antibody, and reduce WB-1 accordingly.
	The primary antibody has non-specific binding or cross-reactivity with the blocking reagent.	Use pretreat A-b (M01052). Customers can also use the Quick Block Optimization Kit to find the best blocking reagent.
	The wash time is too short.	Adding additional washing steps can further decrease background.
	The signal development time is too long.	Reduce the exposure time. If both the signal and background are high, wait for a few minutes for background signal to go down before exposing the film.
	The equipment or reagents have become contaminated.	Use a clean container for each rinse and wash step. Wear gloves and use clean forceps to handle membranes.

Patent Pending.**For Research Use Only.**

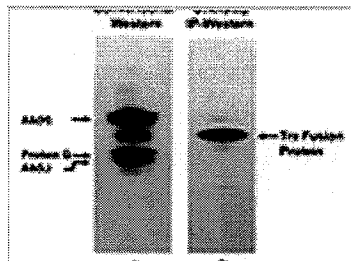
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